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Synthesis and biological evaluation of 3-aryltyramines as fragments binding to BACE-1 and BACE-2

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ABSTRACT

3-Aryltyramines were prepared in one single step from tyramine and various arenediazonium salts by radical arylation. Binding as well as enzyme inhibition data of the 14 compounds do not prove true interaction with BACE-1. In contrast, with BACE-2 inhibition and binding could be confirmed indicating that 3-aryltyramines are potential starting points for a drug discovery effort.

Tyramine[1] and tyrosine[2] derivatives structurally modified by ring-arylation have recently become of interest as new building blocks for pharmaceutical research. In fragment screening experiments, Kuglstatter et al.[1] observed that compounds possessing a (2-hydroxybiphen-5-yl)ethylamine substructure bind to the β-site amyloid precursor protein cleavage enzyme 1 (BACE-1) and act as inhibitors. Due to its essential role in the amyloid cascade hypothesis, BACE-1 today represents one of the most attractive drug targets for the treatment of Alzheimer's disease. According to the hypothesis, BACE-1 is the enzyme responsible for the formation of the pathological 40 or 42 amino acid containing β-amyloid peptides. The cerebral plaques resulting from the deposition of β-amyloid peptides are considered to be responsible for Alzheimer's disease and the related symptoms of dementia.[3,4] To show activity in vivo, potential BACE-1 inhibitors have to be able to pass the blood–brain barrier. For this purpose, molecular properties like low molecular weight and positive charge are beneficial. Both requirements are fulfilled by tyramine (**1**) and its arylated derivatives **2a** and **2b**, which have so far been identified as inhibitors for BACE-1 (Fig. 1).[1] BACE-2, which is structurally a close homolog of BACE-1,[5] is expressed in beta cells and involved in the cleavage of TMEM27. Consequently, inhibition of BACE-2 could be of therapeutic use for the treatment of diabetes.

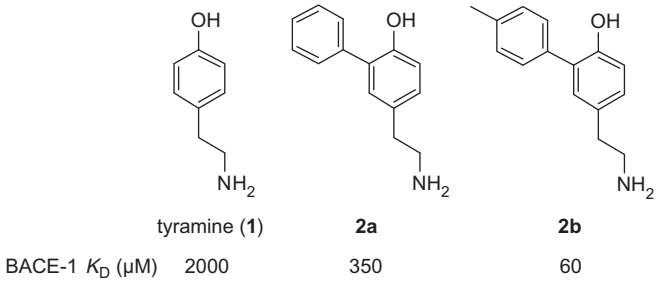

Figure 1. 3-Aryltyramines **2a** and **2b** acting as BACE-1 inhibitors.

Based on the crystal structure of the enzyme,[6] in silico docking experiments have been performed with a number of substituted tyramines.[7] Among the compounds investigated, especially those tyramines bearing a *meta*-substituted aromatic phenyl group as substituent show a good interaction with the active site of BACE-1 with the potential to reach out to the S3 pocket (Fig. 2). This structure based molecular design study encouraged us to synthesize a number of such compounds and test their binding as well as biological activity to BACE-1 and BACE-2.

Our interest in 3-aryltyramines such as **2a** and **2b** was further driven by recent developments in the field of radical arylation of

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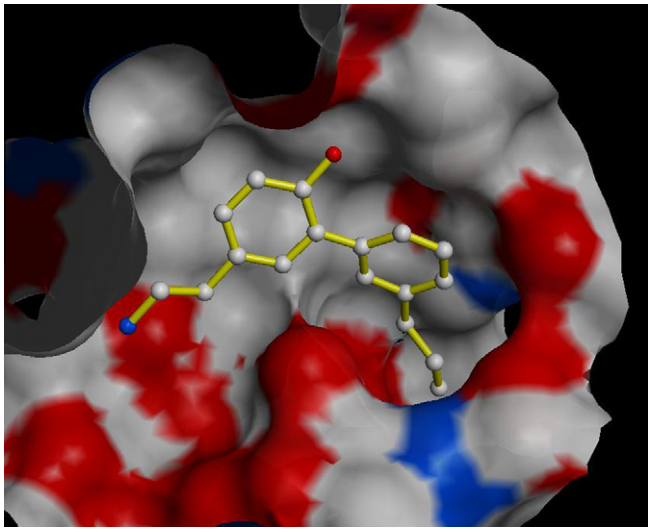


Figure 2. Tyramine derivative with *meta*-substituted phenyl substituent docked to the active site of BACE-1.

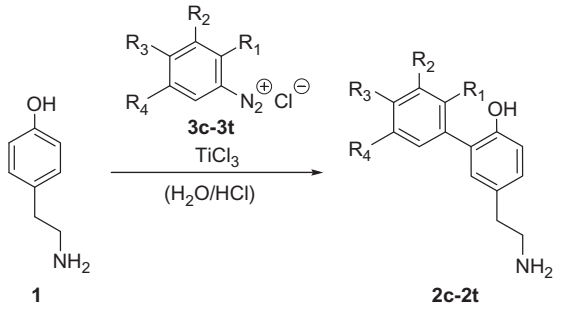
non-protected phenols and anilines.[8–10] Especially through the use of arenediazonium salts,[11] such as **3c**, as aryl radical source, tyramine (**1**) can be converted into its 3-aryl derivative **2c** in a single step without the need for protecting groups (Scheme 1).[10b,12,13] The existing access, which has been exploited for the synthesis of the BACE-1 inhibitors **2a** and **2b**, comprises four steps with a Suzuki coupling as the key reaction.[1]

With the perspective of applying radical arylation reactions in automated parallel syntheses, we decided to evaluate the scope of this new methodology by preparing a small library of 3-aryltyramines in combination with biological testing as BACE-1 and BACE-2 inhibitors. The radical reactions for the library synthesis were carried out using titanium(III)-chloride as the reductant in diluted hydrochloric acid and acetonitrile. Diazonium salts were prepared as chlorides and were added to a solution containing tyramine (**1**) and $TiCl_3$. Since the 3-aryltyramines **2c**–**2t** are less polar than tyramine (**1**), product separation from excess **1** was largely possible by simple extraction of the basified aqueous reaction mixture with diethyl ether.[14] The compounds synthesized through the radical arylation reactions are summarized in Table 1.

Aryltyramines **2c–2k** were accessible without any complications. Products **2l** and **2m** were obtained in lower yields, which are most probably due to the steric hindrance caused by their *ortho*-substituents. In addition, we were unable to fully separate **2l** and **2m** from their regioisomers by HPLC.

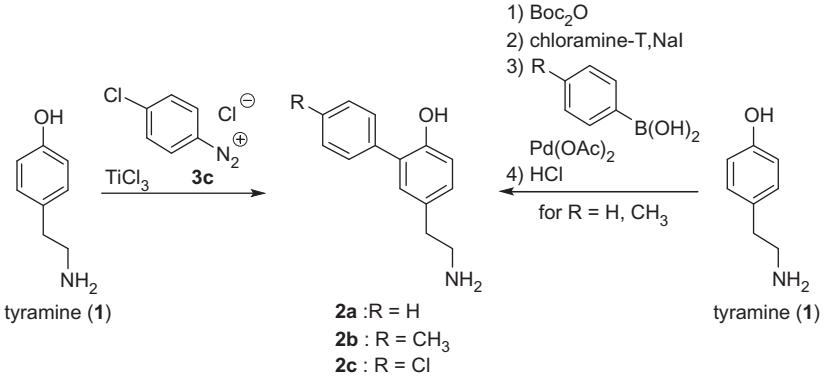
The syntheses starting from the diazonium salts **3n**–**3t** gave the desired products in lower yields, since they were complicated by different side-reactions. Due to its electron-donating substituent, the diazonium salt **3n** was reduced too slowly to effectively give aryl radicals at ambient temperature.[15] In the cases of **3h**, **3k**, **3o**, **3p**, **3r,** and **3s**, additional acetonitrile had to be added to achieve solubility in the diazotization step. Larger amounts of this solvent usually lead to increased hydrogen abstraction.[16,17] The radical arylations with the iodinated diazonium salts **3o** and **3q** gave 1,3- and 1,4-diiodobenzene as by-products, which suggest the occurrence of intramolecular iodine transfer reactions.[18] Significant homocoupling, which means the addition of aryl radicals to their original diazonium salts, was observed in the arylation with salt **3t**. Due to the fact that especially well stabilized cyclohexandienyl radicals (captodative effect) arise from the addition step,[19]

Table 1
3-Aryltyramines **2c–2t** prepared by radical arylation of tyramine (**1**)



Product (diazonium salt)	R^1	R^2	R^3	R^4	Yield[a,b] (%)
2c (**3c**)	H	H	Cl	H	42
2d (**3d**)	H	H	F	H	43
2e (**3e**)	H	Cl	H	H	41
2f (**3f**)	H	F	H	H	40
2g (**3g**)	H	H	Br	H	46
2h (**3h**)	H	Br	H	H	61
2i (**3i**)	H	CN	H	H	35
2j (**3j**)	H	F	F	F	39
2k (**3k**)	H	CF_3	H	H	40
2l (**3l**)	Cl	H	H	H	40[c]
2m (**3m**)	Br	H	H	H	27[c]
2n (**3n**)	H	H	OMe	H	10[d]
2o (**3o**)	H	I	H	H	41[d]
2p (**3p**)	H	Cl	Cl	H	41[d]
2q (**3q**)	H	H	I	H	26[d]
2r (**3r**)	H	H	COMe	H	14[d]
2s (**3s**)	H	H	COOMe	H	7[d]
2t (**3t**)	H	OMe	H	H	22[d]

[a] For reaction conditions, see Experimental section.
[b] Yield after extraction with Et_2O (corrected for minor amounts of **1**, regioisomer and by-products, extraction not quantitative).
[c] Separation of regioisomer not possible by HPLC.
[d] 3-Aryltyramines accompanied by further by-products (see text).



Scheme 1. Radical and organometallic access to 3-aryltyramines **2a**–**2c**.

Table 2
In-vitro binding and inhibition data

Compound	BACE-1 IC_{50}	BACE-1 KD	BACE-2 IC_{50}	BACE-2 KD
2c	>2000	n.d.	>2000	400
2d	>2000	n.d.	1300	600
2e	>2000	n.d.	1300	500
2f	>2000	n.d.	2200	400
2g	>2000	n.d.	2500	400
2h	>2000	n.d.	800	300
2i	>2000	n.d.	1300	300
2j	>2000	n.d.	>2000	300
2k	>2000	n.d.	2000	300
2l	2000	n.d.	>2000	500
2o	>2000	n.d.	500	150
2p	>2000	n.d.	1900	400
2q	>2000	n.d.	1400	n.d.
2t	>2000	n.d.	>2000	500

IC_{50} and K_D values are in μm. n.d. = not determined due to solubility problems, >2000 = no detection of inhibition at 2000 μm. IC_{50} values are the mean of two independent experiments. As the K_m values of the substrates are 28 μm and 35 μm for BACE-1 and BACE-2, respectively, and the concentration used is 300 nm, IC_{50} is about equivalent to K_i[20]K_D's are estimated values as no full saturation of the binding could be observed at concentrations used for the dose response experiments.

homocoupling is now able to compete with the desired addition of the 3-methoxyphenyl radical to tyramine (**1**).

In conclusion, structurally diverse 3-aryltyramines could be obtained through the simple standard procedure without adaption to a particular diazonium salt. If possible in parallel synthesis, slightly modified conditions are recommended for diazonium salts **3n**, **3p**, **3r,** and **3s**.[15,17] From the set of compounds available, 14 aryltyramines (**2c**–**2l**, **2o**–**2q,** and **2t**) were chosen for biological testing. Table 2 summarizes the results observed for in vitro binding data and biochemical enzyme inhibition assays for BACE-1 and BACE-2.

Overall, all data observed suggest no or very weak interaction of the ligands with the active site of the enzyme. For BACE-1 with its less sensitive assay we could not measure binding in the surface plasmon resonance assay due to the solubility problems for any ligand and no inhibition could be detected. The only exception is compound **2l** with some activity at 2 mM. For BACE-2 we could detect some binding as well as inhibitory activity for a number of ligands. Especially **2h** and **2o** are interesting as they show a good correlation of binding and inhibition with the lowest values measured in the SPR and enzymatic assay.

From a preparative point of view, radical arylations of tyramine can provide a fast and simple access to 3-aryltyramines from readily available starting materials. Since the best results were obtained from the reactions with halogen-substituted arenediazonium salts the method represents a valuable complement to known organometallic protocols, which would mostly be difficult to conduct with comparable substitution patterns.[12c] C–H activation of aromatics, as which this type of radical arylation can also be classified, has recently been identified as a priority area for future green chemistry research.[21] Evaluation of the binding and inhibitory activity of the 3-aryltyramines synthesized in the context of this study gives no encouraging results for BACE-1. With the given solubility of the compounds and the sensitivity of the assays an interaction of the ligands with the BACE-1 target protein can not be measured with confidence. On the other hand, for most of the molecules binding activity could be detected against BACE-2. Here, compounds **2h** and **2o** with iodo and bromo substituents in the R^2 position show the lowest IC_{50} and K_D values. Comparison of the data with the unsubstituted 3-phenyltyramine[1] offers a number of interpretations. First, it seems that BACE-1 does not tolerate substitutions at the phenyl ring in any positions indicating a steep structure–activity relationship of this binding pocket. Second, besides the high degree of similarity between the enzymes, BACE-2 can tolerate such substitution much better indicating more flexibility of the pocket. Third, overall BACE-2 shows a better ability for interaction with 3-aryltyramines compared to BACE-1. Consequently, the results reported here are encouraging for the further evaluation of tyramine derivatives for the drug discovery of BACE-2 inhibitors.

Experimental section

General remarks

Solvents and reagents were degassed with nitrogen prior to use in radical reactions. ^{1}H NMR spectra were recorded on 360 and 600 MHz spectrometers using $CDCl_3$ and CD_3OD as solvents referenced to $CHCl_3$ (7.26 ppm) and CHD_2OD (3.31 ppm). Chemical shifts are reported in parts per million (ppm). Coupling constants are in Hertz (*J* Hz). The following abbreviations are used for the description of signals: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quadruplet), m (multiplet). ^{13}C NMR spectra were recorded at 90.6 and 150.9 MHz in $CDCl_3$ and CD_3OD using $CDCl_3$ (77.0 ppm) and CD_3OD (49.5 ppm) as standard. Chemical shifts are given in parts per million (ppm). Purification by preparative HPLC was carried out using Varian 940-LC with a Pursuit XRs C8 column (RP-8, 150 × 21.2 mm, 5 μm particle size) and PDA detection (Varian, 254 and 280 nm).

General procedure for the radical arylation

Preparation of the arenediazonium chloride

To an ice-cooled degassed solution of aniline (5.00 mmol) in 3 N hydrochloric acid (5 mL) and water (5 mL) was dropwise added a degassed solution of sodium nitrite (5.00 mmol, 0.35 g) in water (2.5 mL) over a period of 10 min. After stirring for 20 more minutes at 0 °C, the clear solution was used for the aryl-aryl coupling reactions (5 mmol/12.5 mL = 0.4 M).

Biaryl synthesis

A 5 mL aliquot of the 0.4 M arenediazonium chloride solution (2.00 mmol) was added dropwise by a syringe pump to a vigorously stirred solution of tyramine hydrochloride (6.00 mmol, 1.04 g) in water (6 mL), acetonitrile (4 mL), and titanium(III)-chloride (4.00 mmol, 4.00 mL, ca. 1 M solution in 3 N hydrochloric acid) under nitrogen atmosphere over 10–15 min. After the addition was complete, the mixture was left to stir for 10 more minutes and a solution of sodium hydroxide (2 g) and sodium sulfite (2 g) in water (20 mL) was added. After extraction with diethylether (3 × 75 mL), the combined organic phases were washed with satd. aqueous sodium chloride and dried over sodium sulfate. The solvent was removed under reduced pressure. Prior to purification by HPLC, the crude product was dissolved in dichloromethane (10 mL) and TFA (1.5 equiv) was added. After concentration in vacuo, the trifluoroacetate salt was submitted to preparative HPLC (gradient: $CH_3CN/(H_2O + 0.1\%$ TFA) = 10:90→95:5 over 8 min, flow: 20 mL/min). Additional analytic data obtained from the characterization of the compounds can be found in the Supplementary data.

2-(4′-Chloro-6-hydroxybiphen-3-yl)ethylamine (**2c**): From 4-chloroaniline and tyramine hydrochloride according to the general procedure described above. Purification of the crude product (0.84 mmol, 208 mg, 42%) by preparative HPLC gave 2-(4′-chloro-6-hydroxybiphen-3-yl)ethylamine (**2c**) as a yellow oil: ^{1}H NMR (360 MHz, CD_3OD): δ (ppm) = 2.90 (t, *J* = 7.6 Hz, 2 H), 3.16 (t, *J* = 7.6 Hz, 2 H), 6.89 (d, *J* = 8.2 Hz, 1 H), 7.09 (dd, *J* = 2.3 Hz,

J = 8.2 Hz, 1H), 7,17 (d, *J* = 2.3 Hz, 1 H), 7.37 (d, *J* = 8.6 Hz, 2 H), 7.55 (d, *J* = 8.6 Hz, 2 H); MS (EI): *m*/*z* (%): 248 (2) [M$^+$], 247 (12), 220 (42), 219 (30), 218 (100), 217 (36), 183 (15), 182 (17), 181 (39), 165 (11), 154 (12), 153 (25), 152 (36), 127 (13), 77 (11), 69 (48), 51 (27), 46 (53).

*2-(4′-Fluoro-6-hydroxybiphen-3-yl)ethylamine (**2d**)*: From 4-fluoroaniline and tyramine hydrochloride according to the general procedure described above. Purification of the crude product (0.86 mmol, 199 mg, 43%) by preparative HPLC gave 2-(4′-fluoro-6-hydroxybiphen-3-yl)ethylamine (**2d**) as a brown oil: ^{1}H NMR (360 MHz, CD_3OD): δ (ppm) = 2.89 (t, *J* = 7.7 Hz, 2 H), 3.15 (t, *J* = 7.7 Hz, 2 H), 6.88 (d, *J* = 8.2 Hz, 1 H), 7.07 (dd, *J* = 2.3 Hz, *J* = 8.2 Hz, 1 H), 7.11 (t, J_{HF} = 8.9 Hz, *J* = 8.9 Hz, 2 H), 7.15 (d, *J* = 2.3 Hz, 1 H), 7.56 (dd, J_{HF} = 5.5 Hz, *J* = 8.9 Hz, 2 H); MS (EI): *m*/*z* (%): 231 (18) [M$^+$], 203 (27), 202 (100), 201 (67), 199 (12), 183 (12), 181 (17), 171 (12), 170 (12), 152 (16), 133 (11), 69 (29), 51 (17), 46 (33).

*2-(3′-Chloro-6-hydroxybiphen-3-yl)ethylamine (**2e**)*: From 3-chloroaniline and tyramine hydrochloride according to the general procedure described above. Purification of the crude product (0.82 mmol, 203 mg, 41%) by preparative HPLC gave 2-(3′-chloro-6-hydroxybiphen-3-yl)ethylamine (**2e**) as an orange solid: ^{1}H NMR (360 MHz, CD_3OD): δ (ppm) = 2.90 (t, *J* = 7.6 Hz, 2 H), 3.16 (t, *J* = 7.6 Hz, 2 H), 6.90 (d, *J* = 8.3 Hz, 1 H), 7.10 (dd, *J* = 2.3 Hz, *J* = 8.25 Hz, 1 H), 7.18 (d, *J* = 2.2 Hz, 1 H), 7.30 (ddd, *J* = 1.2 Hz, *J* = 2.1 Hz, *J* = 8.0 Hz, 1 H), 7.36 (t, *J* = 7.8 Hz, 1 H), 7.48 (dd, *J* = 1.8 Hz, *J* = 3.3 Hz, 1 H), 7.58 (dd, *J* = 1.1 Hz, *J* = 2.7 Hz, 1 H); MS (EI): *m*/*z* (%): 248 (5) [M$^+$], 247 (27), 221 (14), 220 (100), 219 (68), 218 (100), 217 (78), 183 (31), 182 (18), 181 (66), 165 (15), 154 (22), 153 (42), 152 (59), 151 (16), 139 (12), 127 (13), 77 (13), 69 (49), 51 (31), 50 (11), 46 (57).

*2-(3′-Fluoro-6-hydroxybiphen-3-yl)ethylamine (**2f**)*: From 3-fluoroaniline and tyramine hydrochloride according to the general procedure described above. Purification of the crude product (0.80 mmol, 185 mg, 40%) by preparative HPLC gave 2-(3′-fluoro-6-hydroxybiphen-3-yl)ethylamine (**2f**) as an orange solid: ^{1}H NMR (360 MHz, CD_3OD): δ (ppm) = 2.91 (t, *J* = 7.6 Hz, 2 H), 3.16 (t, *J* = 7.6 Hz, 2 H), 6.90 (d, *J* = 8.3 Hz, 1 H), 6.99–7.05 (m, 1 H), 7.10 (dd, *J* = 2.3 Hz, *J* = 8.3 Hz, 1 H), 7.19 (d, *J* = 2.3 Hz, 1 H), 7.30–7.34 (m, 1 H), 7.35–7.42 (m, 2 H); MS (EI): *m*/*z* (%): 231 (20) [M$^+$], 203 (37), 202 (100), 201 (87), 199 (13), 183 (15), 181 (30), 171 (14), 170 (17), 152 (23), 146 (13), 133 (15), 69 (43), 51 (26), 50 (10), 46 (50).

*2-(4′-Bromo-6-hydroxybiphen-3-yl)ethylamine (**2g**)*: From 4-bromoaniline and tyramine hydrochloride according to the general procedure described above. Purification of the crude product (0.92 mmol, 269 mg, 46%) by preparative HPLC gave 2-(4′-bromo-6-hydroxybiphen-3-yl)ethylamine (**2g**) as a yellow solid: ^{1}H NMR (360 MHz, CD_3OD): δ (ppm) = 2.90 (t, *J* = 7.6 Hz, 2 H), 3.16 (t, *J* = 7.5 Hz, 2 H), 6.89 (d, *J* = 8.2 Hz, 1 H), 7.09 (dd, *J* = 2.3 Hz, *J* = 8.2 Hz, 1 H), 7.17 (d, *J* = 2.0 Hz, 1 H), 7.48 (d, *J* = 8.8 Hz, 2 H), 7.53 (d, *J* = 8.74 Hz, 2 H); MS (EI): *m*/*z* (%): 292 (3) [M$^+$], 291 (13), 265 (18), 264 (100), 263 (41), 262 (100), 261 (25), 183 (30), 182 (29), 181 (84), 168 (11), 165 (10), 154 (14), 153 (29), 152 (41), 69 (31), 51 (22), 46 (39).

*2-(3′-Bromo-6-hydroxybiphen-3-yl)ethylamine (**2h**)*: From 3-bromoaniline and tyramine hydrochloride according to the general procedure described above. Purification of the crude product (1.22 mmol, 356 mg, 61%) by preparative HPLC gave 2-(3′-bromo-6-hydroxybiphen-3-yl)ethylamine (**2h**) as a brown solid: ^{1}H NMR (360 MHz, CD_3OD): δ (ppm) = 2.90 (t, *J* = 7.6 Hz, 2 H), 3.16 (t, *J* = 7.6 Hz, 2 H), 6.90 (d, *J* = 8.3 Hz, 1 H), 7.10 (dd, *J* = 2.3 Hz, *J* = 8.3 Hz, 1 H), 7.17 (d, *J* = 2.3 Hz, 1 H), 7.30 (t, *J* = 7.9 Hz, 1 H), 7.44 (ddd, *J* = 1.0 Hz, *J* = 2.0 Hz, *J* = 8.0 Hz, 1 H), 7.53 (ddd, *J* = 1.1 Hz, *J* = 1.6 Hz, *J* = 7.7 Hz, 1 H), 7.7 (t, *J* = 7.6 Hz, 1 H); MS (EI): *m*/*z* (%): 293 (8) [M$^+$+H], 291 (10), 265 (15), 264 (100), 263 (36), 262 (100), 261 (21), 183 (30), 182 (27), 181 (76), 165 (10), 154 (14), 153 (26), 152 (37), 69 (33), 51 (22), 46 (39).

*2-(3′-Cyano-6-hydroxybiphen-3-yl)ethylamine (**2i**)*: From 3-cyanoaniline and tyramine hydrochloride according to the general procedure described above. Purification of the crude product (0.70 mmol, 167 mg, 35%) by preparative HPLC gave 2-(3′-cyano-6-hydroxybiphen-3-yl)ethylamine (**2i**) as an orange solid: ^{1}H NMR (360 MHz, CD_3OD): δ (ppm) = 2.91 (t, *J* = 7.6 Hz, 2 H), 3.17 (t, *J* = 7.6 Hz, 2 H), 6.92 (d, *J* = 8.3 Hz, 1 H), 7.14 (dd, *J* = 2.3 Hz, *J* = 8.3 Hz, 1 H), 7.21 (d, *J* = 2.2 Hz, 1 H), 7.57 (t, *J* = 7.8 Hz, 1 H), 7.65 (td, *J* = 1.3 Hz, *J* = 7.7 Hz, 1 H), 7.89 (ddd, *J* = 1.2 Hz, *J* = 1.7 Hz, *J* = 7.9 Hz, 1 H), 7.93 (t, *J* = 1.4 Hz, 1 H); MS (EI): *m*/*z* (%): 238 (16) [M$^+$], 210 (16), 209 (100), 208 (34), 153 (12), 152 (15), 151 (9), 77 (9), 69 (21), 51 (15), 46 (24).

*2-(3′,4′,5′-Trifluoro-6-hydroxybiphen-3-yl)ethylamine (**2j**)*: From 3,4,5-trifluoroaniline and tyramine hydrochloride according to the general procedure described above. Purification of the crude product (0.78 mmol, 208 mg, 39%) by preparative HPLC gave 2-(3′,4′,5′-trifluoro-6-hydroxybiphen-3-yl)ethylamine (**2j**) as an orange solid: ^{1}H NMR (600 MHz, CD_3OD): δ (ppm) = 2.90 (t, *J* = 7.6 Hz, 2 H), 3.16 (t, *J* = 7.6 Hz, 2 H), 6.90 (d, *J* = 8.3 Hz, 1 H), 7.12 (dd, *J* = 2.3 Hz, *J* = 8.3 Hz, 1 H), 7.20 (d, *J* = 2.2 Hz, 1 H), 7.26 (dd, J_{HF} = 6.8 Hz, J_{HF} = 9.4 Hz, 2 H); MS (EI): *m*/*z* (%): 267 (12) [M$^+$], 239 (14), 238 (100), 237 (44), 219 (9), 217 (9), 189 (11), 188 (17), 169 (14), 77 (9), 69 (41), 51 (25), 50 (9), 46 (44).

*2-(3′-Trifluoromethyl-6-hydroxybiphen-3-yl)ethylamine (**2k**)*: From 4-(3-trifluoromethyl)aniline and tyramine hydrochloride according to the general procedure described above. Purification of the crude product (0.80 mmol, 225 mg, 40%) by preparative HPLC gave 2-(3′-trifluoromethyl-6-hydroxybiphen-3-yl)ethylamine (**2k**) as a beige solid: ^{1}H NMR (600 MHz, CD_3OD): δ (ppm) = 2.92 (t, *J* = 7.6 Hz, 2 H), 3.17 (t, *J* = 7.6 Hz, 2 H), 6.92 (d, *J* = 8.3 Hz, 1 H), 7.13 (dd, *J* = 2.3 Hz, *J* = 8.3 Hz, 1 H), 7.21 (d, *J* = 2.2 Hz, 1 H), 7.56–7.60 (m, 2 H), 7.80–7.83 (m, 1 H), 7.87 (s, 1 H); MS (EI): *m*/*z* (%): 281 (9) [M$^+$], 262 (9), 252 (100), 251 (16), 231 (28), 183 (18), 181 (10), 152 (11), 69 (23), 51 (13), 46 (28).

*2-(2′-Chloro-6-hydroxybiphen-3-yl)ethylamine (**2l**)*: From 2-chloroaniline and tyramine hydrochloride according to the general procedure described above. Purification of the crude product (0.80 mmol, 198 mg, 40%) by preparative HPLC gave a mixture of the regioisomers 2-(2′-chloro-6-hydroxybiphen-3-yl)ethylamine (**2l**) and 2-(2′-chloro-5-hydroxybiphen-2-yl)ethylamine (ratio 5:1) as a brown oil: ^{1}H NMR (360 MHz, CD_3OD, major isomer): δ (ppm) = 2.89 (t, *J* = 7.6 Hz, 2 H), 3.14 (t, *J* = 7.6 Hz, 2 H), 6.89 (d, *J* = 8.3 Hz, 1 H), 7.01 (d, *J* = 2.2 Hz, 1 H), 7.13 (dd, *J* = 2.4 Hz, *J* = 8.3 Hz, 1 H), 7.28–7.31 (m, 3 H), 7.43–7.46 (m, 1 H); ^{1}H NMR (360 MHz, CD_3OD, minor isomer): δ (ppm) = 2.63–2.75 (m, 2 H), 2.84–2.93 (m, 2 H), 6.58 (d, *J* = 2.6 Hz, 1 H), 6.83 (dd, *J* = 2.6 Hz, *J* = 8.4 Hz, 1 H), 7.19 (d, *J* = 8.6 Hz, 1 H), 7.27–7.30 (m, 1 H), 7.38 (dd, *J* = 3.6 Hz, *J* = 5.7 Hz, 2 H), 7.49–7.52 (m, 1 H).

*2-(2′-Bromo-6-hydroxybiphen-3-yl)ethylamine (**2m**)*: From 2-bromoaniline and tyramine hydrochloride according to the general procedure described above. Purification of the crude product (0.54 mmol, 158 mg, 27%) by preparative HPLC gave a mixture of the regioisomers 2-(2′-bromo-6-hydroxybiphen-3-yl)ethylamine (**2m**) and 2-(2′-bromo-5-hydroxybiphen-2-yl)ethylamine (ratio 3:1) as a brown oil: ^{1}H NMR (360 MHz, CD_3OD, major isomer): δ (ppm) = 2.90 (t, *J* = 7.6 Hz, 2 H), 3.14 (t, *J* = 7.6 Hz, 2 H), 6.88 (d, *J* = 8.3 Hz, 1 H), 6.99 (d, *J* = 2.3 Hz, 1 H), 7.13 (dd, *J* = 2.3 Hz, *J* = 8.3 Hz, 1 H), 7.21 (ddd, *J* = 1.8 Hz, *J* = 7.4 Hz, *J* = 8.0 Hz, 1 H), 7.28–7.30 (m, 1 H), 7.35 (dt, *J* = 1.2 Hz, *J* = 7.4 Hz, 1 H), 7.64 (dd, *J* = 1.0 Hz, *J* = 8.0 Hz, 1 H); ^{1}H NMR (360 MHz, CD_3OD, minor isomer): δ (ppm) = 2.62–2.78 (m, 2 H), 2.83–2.98 (m, 2 H), 6.57 (d, *J* = 2.6 Hz, 1 H), 6.84 (dd, *J* = 2.6 Hz, *J* = 8.4 Hz, 1 H), 7.18 (d, *J* = 8.3 Hz, 1 H), 7.27–7.29 (m, 2 H), 7.42 (dd, *J* = 1.2 Hz, *J* = 7.6 Hz, 1 H), 7.69 (ddd, *J* = 0.7 Hz, *J* = 1.1 Hz, *J* = 7.9 Hz, 1 H).

*2-(4′-Methoxy-6-hydroxybiphen-3-yl)ethylamine (**2n**)*: From 4-methoxyaniline and tyramine hydrochloride according to the general procedure described above. Purification of the crude product (0.20 mmol, 49 mg, 10%) by preparative HPLC gave 2-(4′-methoxy-6-hydroxybiphen-3-yl)ethylamine (**2n**) as a brown oil: ^{1}H NMR (360 MHz, CD_3OD): δ (ppm) = 2.89 (t, J = 7.6 Hz, 2 H), 3.15 (t, J = 7.6 Hz, 2 H), 3.82 (s, 3 H), 6.87 (d, J = 8.2 Hz, 1 H), 6.94 (d, J = 8.9 Hz, 2 H), 7.03 (dd, J = 2.3 Hz, J = 8.2 Hz, 1 H), 7.14 (d, J = 2.3 Hz, 1 H), 7.48 (d, J = 8.9 Hz, 2 H); MS (EI): m/z (%): 243 (14) [M^+], 215 (16), 214 (100), 213 (31), 199 (13), 141 (11), 137 (14), 120 (14), 115 (11), 108 (81), 107 (55), 91 (10), 77 (31), 69 (61), 51 (36), 50 (12), 46 (67), 45 (10).

*2-(3′-Iodo-6-hydroxybiphen-3-yl)ethylamine (**2o**)*: From 3-iodoaniline and tyramine hydrochloride according to the general procedure described above. Purification of the crude product (0.82 mmol, 278 mg, 41%) by preparative HPLC gave 2-(3′-iodo-6-hydroxybiphen-3-yl)ethylamine (**2o**) as a red-brown oil: ^{1}H NMR (360 MHz, CD_3OD): δ (ppm) = 2.91 (t, J = 7.6 Hz, 2 H), 3.16 (t, J = 7.6 Hz, 2 H), 6.90 (d, J = 8.2 Hz, 1 H), 7.09–7.17 (m, 3 H), 7.56 (ddd, J = 1.1 Hz, J = 1.7 Hz, J = 7.8 Hz, 1 H), 7.63 (ddd, J = 1.0 Hz, J = 1.8 Hz, J = 7.9 Hz, 1 H), 7.94 (t, J = 1.6 Hz, 1 H); MS (EI): m/z (%): 339 (10) [M^+], 311 (24), 310 (100), 309 (14), 183 (37), 182 (40), 181 (74), 168 (14), 165 (10), 153 (22), 152 (36), 115 (10), 77 (11), 76 (11), 69 (40), 51 (27), 46 (45).

*2-(3′,4′-Dichloro-6-hydroxybiphen-3-yl)ethylamine (**2p**)*: From 3,4-dichloroaniline and tyramine hydrochloride according to the general procedure described above. Purification of the crude product (0.82 mmol, 231 mg, 41%) by preparative HPLC gave 2-(3′,4′-dichloro-6-hydroxybiphen-3-yl)ethylamine (**2p**) as a brown solid: ^{1}H NMR (600 MHz, CD_3OD): δ (ppm) = 2.90 (t, J = 7.6 Hz, 2 H), 3.16 (t, J = 7.6 Hz, 2 H), 6.90 (d, J = 8.3 Hz, 1 H), 7.11 (dd, J = 2.3 Hz, J = 8.3 Hz, 1 H), 7.19 (d, J = 2.1 Hz, 1 H), 7.49–7.53 (m, 2 H), 7.75 (dd, J = 0.4 Hz, J = 1.2 Hz, 1 H); MS (EI): m/z (%): 283 (7) [M^++H], 181 (11), 256 (11), 255 (12), 254 (62), 253 (29), 252 (100), 251 (26), 217 (12), 215 (19), 182 (11), 181 (24), 153 (20), 152 (42), 151 (13), 69 (25), 51 (17), 46 (29).

*2-(4′-Iodo-6-hydroxybiphen-3-yl)ethylamine (**2q**)*: From 4-iodoaniline and tyramine hydrochloride according to the general procedure described above. Purification of the crude product (0.52 mmol, 176 mg, 26%) by preparative HPLC gave 2-(4′-iodo-6-hydroxybiphen-3-yl)ethylamine (**2q**) as a yellow oil: ^{1}H NMR (600 MHz, CD_3OD): δ (ppm) = 2.90 (t, J = 7.6 Hz, 2 H), 3.12 (t, J = 7.6 Hz, 2 H), 6.90 (d, J = 8.2 Hz, 1 H), 7.04 (dd, J = 2.3 Hz, J = 8.2 Hz, 1 H), 7.09 (d, J = 2.3 Hz, 1 H), 7.33 (d, J = 8.5 Hz, 2 H), 7.72 (d, J = 8.5 Hz, 2 H); MS (EI): m/z (%): 339 (9) [M^+], 311 (14), 310 (100), 309 (10), 183 (25), 182 (29), 181 (56), 168 (13), 153 (17), 152 (25), 69 (35), 51 (24), 46 (43).

*2-(3′-Methoxy-6-hydroxybiphen-3-yl)ethylamine (**2t**)*: From 3-methoxyaniline and tyramine hydrochloride according to the general procedure described above. Purification of the crude product (0.44 mmol, 107 mg, 22%) by preparative HPLC gave 2-(3′-methoxy-6-hydroxybiphen-3-yl)ethylamine (**2e**) as a brown oil: ^{1}H NMR (360 MHz, CD_3OD): δ (ppm) = 2.90 (t, J = 7.6 Hz, 2 H), 3.15 (t, J = 7.6 Hz, 2 H), 3.81 (s, 3 H), 6.85 (ddd, J = 1.0 Hz, J = 2.6 Hz, J = 8.3 Hz, 1 H), 6.89 (d, J = 8.3 Hz, 1 H), 7.07 (dd, J = 2.3 Hz, J = 8.2 Hz, 1 H), 7.09–7.12 (m, 1 H), 7.12 (d, J = 1.5 Hz, 1 H), 7.17 (d, J = 2.2 Hz, 1 H), 7.29 (dt, J = 1.3 Hz, J = 8.2 Hz, 1 H).

Surface plasmon resonance (SPR) assay

The SPR assay was performed on a Biacore T200 instrument using CM5 sensors. The proteins were immobilized using standard amine coupling chemistry. Acetate buffer (10 mM acetate pH 4.6, 150 mM NaCl, 3 mM EDTA, 0.01% P20) was used as running buffer during the immobilization. The proteins (50 μg/mL) were dissolved in acetate buffer (10 mM acetate, pH 4.6) during immobilization. 12000 RU's and 6000 RU's of protein were immobilized in case of BACE-1 and BACE-2, respectively. Acetate buffer (10 mM acetate, pH 4.6, 150 mM NaCl, 3 mM EDTA, 0.01% P20, 4% DMSO) was used as the running buffer during binding experiments. Compounds were dissolved and diluted in pure DMSO and these DMSO solutions added to acetate buffer to obtain buffer solutions of the compounds of desired concentration. The immobilized BACE-1 and BACE-2 were contacted with these solutions and the response at the end of the injection phase after solvent correction taken as the measure for compound binding. These responses were used to estimate the K_D values.

Enzyme inhibition assay

The fluorescent substrate human BACE-1 and BACE-2 kinetic assays were performed at room temperature in 384-well microtiter plates (black with clear flat bottom, non binding surface plates from Corning, Cat. No.: 3655) in a final volume of 51 μL. The recombinant enzymes (BACE-1 expressed in SF9 cells and BACE-2 in E. coli and purified at F. Hoffmann-La Roche, Basel) and the substrate were diluted in assay buffer (100 mM sodium acetate, 20 mM EDTA, 0.05% BSA, pH 4.5). Tyramines 100 mM DMSO stock solutions were serially diluted in DMSO (15 concentrations, 1/3 dilution steps; final concentration range: 2000–0.0004 μM) and 1 μL of diluted compounds were mixed for 10 min with 40 μL of BACE-1 (final concentration: 30 nM) or with 40 μL of BACE-2 (final concentration: 125 nM). After addition of 10 μL of the substrate WSEVNLDAEFRC-MR121 (final concentration: 300 nM), the plates were strongly shaked for 2 min. The enzymatic reaction was followed in a plate vision reader (Perkin–Elmer) (excitation wavelength: 630 nm; emission: 695 nm) for at least 30 min in a kinetic measurement detecting an increase of MR121 fluorescence during the reaction time. The slope in the linear range of the kinetic was calculated and the IC_{50} of the test compounds were determined using a four parameter equation for curve fitting.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2012.02.070.

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